(2)

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER / 2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) **Sporicidal Activity of Alcide Exspor and Sodium Hypochlorite on Bacillus anthracis spores.**	5. TYPE OF REPORT & PERIOD COVERED
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) **John W. Ezzell, Jr.**	8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS **Bacteriology Division SGRD-UIB USAMRIID Fort Detrick, Frederick, MD 21701-5011**	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS	12. REPORT DATE
	13. NUMBER OF PAGES
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report)
	15a. DECLASSIFICATION/ DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

Approved for public release; distribution unlimited

18. SUPPLEMENTARY NOTES

To be published in Applied and Environmental Microbiology

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Alcide, Exspor, spore, Bacillus anthracis, Anthrax, Sodium hypochlorite, disinfectant, decontamination, infectious diseases

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Exspor, a commercial disinfectant, was completely sporicidal to Bacillus anthracis spores and was active in the presence of 5% (wt/vol) brain heart infusion, 5% (vol/vol) whole sheep blood, and 50% (wt/vol) plant materials. Sodium hypochlorite, 0.5% (wt/vol), although highly sporicidal, was inhibited significantly by the presence of organic material.

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Sporicidal Activity of Alcide Exspor™ and Sodium Hypochlorite on *Bacillus anthracis* spores.

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Cleared for Publication: 14 October 1986

Exspor™, a commercial disinfectant, was completely sporicidal to Bacillus anthracis spores and was active in the presence of 5% (wt/vol) brain heart infusion, 5% (vol/vol) whole sheep blood, and 50% (wt/vol) plant materials. Sodium hypochlorite, 0.5% (wt/vol), although highly sporicidal, was inhibited significantly by the presence of organic material.

Bacillus anthracis, a Gram-positive, endospore-forming bacterium, is the etiologic agent of anthrax, a disease primarily associated with herbivores. Its spores present special problems with respect to decontamination and control in infected herds and in laboratories because they are resistant to many common disinfectants. Although sodium hypochlorite (household bleach), formaldehyde, and phenol are effective sporicidal decontaminants, they suffer the disadvantage of being caustic and corrosive in addition to being toxic and offensive to humans and animals.

I performed studies on a new commercial sporicidal product, Exspor™ (Alcide Corp., Westport, Conn.), to determine its effectiveness in killing B. anthracis spores in solution and on surfaces. Its active ingredient, sodium chlorite, is activated, just prior to use, by the addition of a lactic acid solution provided with the product. The chlorite anion reacts with hydrogen ions to form chlorous acid, which disproportionates to form chlorine dioxide. According to the manufacturer, both the chlorous acid and chlorite generated by Exspor™ are active against a wide variety of bacteria, bacterial spores, fungi, viruses, and parasites, while being non-toxic, non-carcinogenic, and nonmutagenic to humans. Exspor™ has been shown to be effective in treating dermatomycosis in mice (Boyer, J. M., and H. Alliger. 1983. Abstr. Ann. Meet. Am. Soc. Microbiol. F30.) and in inactivating oocysts of certain species of Eimeria (2).

Bacillus anthracis strains, Vollum 1-B and New Hampshire, were obtained from the culture collection at the U.S. Army

Medical Research Institute of Infectious Diseases, Fort Detrick, Md., and were cultured on blood agar at 37°C for 18 to 20 h. Growth from blood agar cultures was inoculated into cotton-plugged, liter flasks containing 100 ml Schaeffer's sporulation medium as modified by Leighton and Doi (1) and shaken at 80 reciprocations per min for 24 h at 37°C. Cultures were incubated an additional 24 h at ambient temperatures. The cultures were inspected by phase microscopy to estimate the degree of sporulation. Once sufficiently sporulated (≥ 99%), the cultures were harvested by centrifugation at 10,000 X g, 15 min. No attempt was made to remove remaining vegetative cells. Spores were quantitated under phase microscopy by using a Petroff-Hauser counting chamber and were adjusted to a concentration of 10^9 spores per ml in nutrient broth (NB, Difco, Detroit, Mich.) supplemented with 10% (vol/vol) glycerol (final concentration). Spore preparations were stored at -20°C.

Sporicidal activity of Exspor™ in solution was tested at three concentrations in the presence and absence of 5% (wt/vol) organic load [brain heart infusion (Difco)]. Tests were performed in open 1.5-ml polypropylene tubes containing 1-ml of test mixture (10^8 spores per ml) and incubated at ambient temperatures. At time intervals ranging from 10 min to 18 h, 0.1 ml of the test mixtures was diluted 1:10 in 0.9 ml of 5% brain heart infusion and 0.4 ml plated in duplicate on blood agar. After 18 to 20 h incubation at 37°C, the cultures were scored "+" or "-" to indicate growth of surviving spores or no

growth, respectively. Since the results on the duplicate plates and the response of both strains were generally identical, the data in Table 1 represent the overall effect of the disinfectants.

Exspor™, when used at the manufacturer's recommended concentration (henceforth designated as 1X), was completely effective at 10 min in killing 100% of the spores, even in the presence of the 5% organic load. The disinfectant at 0.5 and 0.1X concentrations required between 60 to 120 min and overnight contact concentrations respectively, to be effective in the presence of 5% BHI. Since I was only interested in sterilization of the test solutions, I made no note was made of partial killing of the spore population. To assure that dilution of the test mixtures in BHI and plating on BA was sufficient to neutralize the disinfectant, spores were mixed with 1X Exspor™ and immediately diluted in BHI and plated on BA. In all cases, the control plates had almost confluent growth, indicating that disinfectant carried over during the procedure was being sufficiently diluted and neutralized.

I tested Exspor™ in conjunction with 0.5% sodium hypochlorite for its sporicidal activity on surfaces. Spore suspensions were spread over the surface of sterile microscope slides (10^6 spores per slide) and allowed to dry overnight in a laminar flow hood at ambient temperatures. Testing was performed by rapidly dipping the slides in disinfectant and immediately placing them, under aseptic conditions, on a sterile towel to

drain. At time intervals, ranging from 0.5 to 120 min, the reaction was stopped by placing treated slides (in duplicate) into tubes containing 45 ml sterile nutrient broth to neutralize the effect of the disinfectants. The tubes containing the slides were capped, incubated at 37°C for 18 to 20 h, and then scored for growth or no growth as described above. The contents of turbid tubes were plated on blood agar to confirm that the turbidity noted was due to surviving spores and not to contamination. Demonstration that residual disinfectant on the slide was sufficiently diluted and neutralized by the nutrient broth was accomplished by incubating untreated spore slides in nutrient broth along with plain slides that had been dipped in the disinfectants as described above. As shown in Table 2, Exspor™ was sporicidal by 1 min. There was no growth at 30 sec when 0.5% bleach was used. Growth derived from the slides exposed to Exspor™ for only 30 sec clearly showed that the spores remained adhered to the slides during the rapid dipping process. Subsequent studies showed that spores rapidly dipped in 0.5% bleach also remained bound to the surface of the slides. Therefore, I concluded that the lack of growth was due to the sporicidal activity of the tested solutions and not to release of spores from the slides.

Animals dying from anthrax often bleed from their body orifices just prior to death, thereby contaminating the soil or bedding. To control the spread of disease, attending personnel must decontaminate the area after removing the carcass. There-

fore, Exspor™ was compared with 0.5% sodium hypochlorite bleach for sporicidal activity in the presence of 50% (vol/vol) whole sheep blood and in the presence of plant material (i.e., grass, leaves, etc.). Blood containing 10^8 spores per ml was mixed with an equal volume of either 2X Exspor™ or 1% sodium hypochlorite (final concentrations were 1X and 0.5%, respectively). At various time intervals, 0.1-ml samples were plated in duplicate on blood agar. Although Exspor™ killed most of the spores within 1 h, a small percentage of the spores remained viable after 18 h incubation. Exspor™ had a profound effect on the consistency of the blood in that 5 min after to its addition, the blood became a thick, almost solid paste. I suggest that spores entrapped in the solid material were afforded protection from Exspor™. Although 0.5% sodium hypochlorite did not solidify the the blood, it was ineffective in killing spores after overnight incubation. Both disinfectants were effective when their ratio to the blood was increased four-fold or, in the case of bleach, when the concentration was increased three- to four-fold. To test their effectiveness in the presence of plant material, I collected a mixture of grass, leaves, and weeds at random from a wooded area at Fort Detrick. Subsequent to addition of distilled H_2O, the material was homogenized in a high speed blender for 5 min at ambient temperatures to obtain a slurry. Spores were added to 10^8 per ml, mixed, and diluted in an equal volume of either 2X Exspor™ or 1% bleach. Exspor™ was completely sporicidal within 1 h, whereas the bleach did not sterilize the

mixture until 180 min. However, when I tested the disinfectants using the thick plant material which settled out of the slurry, Exspor™ was still 100% sporicidal by 1 h, whereas the bleach was ineffective. The bleach required excessive amounts and higher concentrations to be effective. Data from these studies clearly indicate that the Alcide product is sporicidal, both in solution and on surfaces. The advantages of Exspor™ over the sodium hypochlorite bleach are that it is less corrosive, not caustic, and, according to the manufacturer, is generally not harmful to humans. However, inhalation of aerosolized vapors during decontamination of an enclosed area with Exspor™ may result in breathing difficulties due to the acidity of the solution (Alcide Corp. representative personal communication). It appears that inhibition of Exspor™ activity is due primarily to mechanical reasons in which the disinfectant is physically prevented from making contact with the spores (i.e., spores entrapped in dried blood). Nevertheless, the data presented in this report clearly demonstrate the effectiveness of Exspor™ for sterilization of equipment or areas contaminated with B. anthracis spores. Conversely, there was a high chlorine demand for the hypochlorite caused by the nonspecific interaction with organic matter, thereby requiring larger amounts and higher concentrations to overcome this neutralizing effect on its sporicidal activity. These results are consistent with previous studies (S. N. Spiegelman and C. J. Glambrone, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, Q24, p. 288) demonstrating that hypochlorite lost

most of its cidal activity towards Staphylococcus aureus in the presence of 20 or 40% serum, whereas Alcide LD™ 10:1:1, a product which is essentially identical to Exspor™, retained its cidal activity under the same conditions. Therefore, the amount of sodium hypochorite required to completely sterilize an area is rather subjective in that it is based on the quantity of organic matter present. Therefore, one should never assume an area is decontaminated until it has been checked by culture.

ACKNOWLEDGEMENTS

I thank Teresa Abshire and Anastacio Rosa for their technical assistance. Drs. Bruce Ivins, Gregory Knudson, Martin Crumrine, and Ms. Kathy Kenyon are also thanked for their critiques of this manuscript.

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TABLE 1. Sporicidal activity of Exspor™ in solution with and without organic load.

	Exspor™						Control	
	1X		0.5X		0.1X			
	dH_2O	BHI[a]	dH_2O	BHI	dH_2O	BHI	dH_2O	BHI
10 min	-	-	-	+	-	+	+	+
60 min	-	-	-	+/-[b]	-	+	+	+
120 min	-	-	-	-	-	+	+	+
18 h	-	-	-	-	-	-	+	+

[a] 5% brain heart infusion.

[b] One of the duplicate blood agar cultures had growth and the other had no growth.

TABLE 2. Sporicidal activity of Exspor™ and sodium hypochlorite on spores bound to slides.

	Length of Exposure (min)							
Disinfectant	0.5	1	2.5	5	30	60	120	Control
Exspor™	+	-	-	-	-	-	-	+
Hypochlorite	-	-	-	-	-	-	-	+